REMARKS

Claim 3 is pending in the instant application. Claim 3 has been rejected. The sequence listing of the specification has been amended. No new matter is added by this amendment. Reconsideration is respectfully requested in light of the following remarks and amendments to the sequence listing of the instant application.

I. Amendment to Sequence Listing

Applicants are providing herewith a replacement paper and CRF copy of an amended Sequence Listing inclusive of SEQ ID NO:5. A statement in accordance with 37 C.F.R. 1.821 is also provided herewith. SEQ ID NO:5 is identical to SEQ ID NO:1 set forth in provisional U.S. Patent Application Serial No. 60/166,818 from which the instant case claims priority. Thus, no new matter is added by this amendment.

II. Rejection of Claim 3 under 35 U.S.C. § 112, second paragraph

Claim 3 has been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner suggests that the metes and bounds of the phrase "under stringent

conditions" are not clear.

Applicants respectfully disagree.

In accordance with MPEP § 2173, the primary purpose of the requirement of definiteness of claim language is to ensure that the scope of the claimed is clear so that the public is informed of the boundaries of what constitutes infringement of the patent. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:

(A) The content of the particular application disclosure;

(B) The teachings of the prior art; and

(C) The claim interpretation that would be given by one possessing the ordinary level of skill in that pertinent art at the time the invention was made. Only a reasonable degree of particularly and distinctness is required. MPEP § 2173.01.

As pointed out previously, methods for assessing whether a polynucleotide hybridizes under stringent conditions to a selected polynucleotide sequence are well known to those of skill in the art and set forth in great detail in standard reference texts such as Sambrook et al. 1989 (Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor). Such methods can be performed routinely by those of skill in the art to assess whether or not a polynucleotide

hybridizes under stringent conditions to, for example SEQ ID NO:1 and thus falls within the scope of the claimed polynucleotides.

Further, Applicants have amended the instant specification to include an exemplary polynucleotide sequence, SEQ ID NO:5, which hybridizes under stringent condition to the antisense of SEQ ID NO:1. Support for this amendment is provided in provisional U.S. Patent Application Serial No. 60/166,818 from which the instant case claims priority. Thus, no new matter is added by this amendment.

Thus, the metes and bounds of claim 3 are clear to the skilled artisan when read in light of the prior art and the teachings of the instant specification.

Withdrawal of this rejection under 35 U.S.C. § 112, first paragraph is therefore respectfully requested.

III. Rejection of Claim 3 under 35 U.S.C. § 112, first paragraph - Written Description

Claim 3 has been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. In particular, the Examiner suggests that there is a lack of written description for Ovr107 comprising a polynucleotide which hybridizes under stringent conditions to the antisense sequence of SEQ ID NO:1.

Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended the sequence listing to include SEQ ID NO:5. SEQ ID NO:5 is identical to SEQ ID NO:1 disclosed in provisional U.S. Patent Application Serial No. 60/166,818 from which the instant case claims priority. Thus, no new matter is added by this amendment.

SEQ ID NO:5 is a polynucleotide which hybridizes under stringent conditions to the antisense sequence of SEQ ID NO:1. Thus, the specification, as amended contains a written description evidencing applicants' clear possession of a polynucleotide hybridizing under stringent conditions to the antisense of SEQ ID NO:1 as claimed. Further, the instant specification and its teachings clearly place the public in possession of these sequences as well.

Thus, the instant specification, as amended, meets the "essential goal" of the written description requirements of 35 U.S.C. § 112, first paragraph as set forth in MPEP § 2163.

Withdrawal of this rejection under 35 U.S.C. § 112, first paragraph is therefore respectfully requested.

IV. Rejection of Claim 3 under 35 U.S.C. § 112, first paragraph - Lack of Enablement

Claim 3 has been rejected under 35 U.S.C. § 112, first

paragraph for lack of enablement. The Examiner has acknowledged the specification to be enabling for detection in cells and tissue sample. However, the Examiner suggests that the specification does not reasonably provide enablement for detection in bodily fluids.

Applicants respectfully traverse this rejection.

At the outset, Applicants respectfully disagree with the Examiner suggestion that the art, as a whole do not teach a nucleic acid overexpression would be detected in bodily fluid. Contrary to the Examiner's suggestion, a quick search of the relevant art revealed multiple prior art and contemporary references disclosing detection of cancer markers in various bodily fluids. Applicants are providing a copy of their search results herewith for the Examiner's convenience. These Abstracts of clearly supportive of polynucleotides such as SEQ ID NO:1 being detectable in bodily fluids.

Further, MPEP § 2164.01 states that as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claimed invention, then the enablement requirement of 35 U.S.C. § 112, is satisfied. Exemplary bodily fluids used routinely by those of skill in the art are taught at

page 14, lines 6-10 of the specification. Further, methods for detecting the claimed polynucleotides are taught in detail at page 12, line 15 through page 13, line 16. Thus, contrary to the Examiner's suggestion that the specification lacks guidance, teachings of the specification clearly disclose multiple methods for making and using the claimed invention and bear a reasonable correlation to the entire scope of the claimed. Further, vague suggestions by the Examiner based upon on a clearly erroneous characterization of the predictability in this art field provide no reason to doubt the objective truth of teachings of the specification regarding detectability of this cancer marker in bodily fluid.

Thus, the instant specification clearly meets the enablement requirements of 35 U.S.C. § 112, first paragraph and withdrawal of this rejection is respectfully requested.

V. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending

claims is earnestly solicited.

Respectfully submitted,

Kathleen A. Tyrrell
Registration No. 38,350

Date: **May 13, 2004**

Licata & Tyrrell P.C.
66 E. Main Street
Marlton, New Jersey 08053

(856) 810-1515

Sputum/Lung cancer

Int J Cancer. 2001 Apr 1;92(1):1-8. Related Articles, Links

Sensitive detection of rare cancer cells in sputum and peripheral blood samples of patients with lung cancer by preproGRP-specific RT-PCR.

Lacroix J, Becker HD, Woerner SM, Rittgen W, Drings P, von Knebel Doeberitz M.

Division of Molecular Diagnostics and Therapy, Department of Surgery, University of Heidelberg, Heidelberg, Germany.

RT-PCR-based amplification of transcripts expressed in cancer but not in normal non-neoplastic cells is increasingly used for the sensitive detection of rare disseminated or exfoliated cancer cells to improve cancer staging and early detection protocols. However, these assays are frequently hampered by false-positive test results due to low-level transcription of the marker genes in normal cells. To overcome these limitations, target transcripts have to be identified that are tightly suppressed in normal non-neoplastic tissues, whereas they should be actively transcribed in the respective cancer cells. Here, we tested RT-PCR assays for 7 neuroendocrine marker transcripts including NCAM, PGP 9.5, gastrin, gastrin receptor, synaptophysin, preprogastrin-releasing peptide (preproGRP) and GRP-receptor to detect rare exfoliated tumor cells in peripheral venous blood and sputum samples from patients with lung cancer. Among these preproGRP RT-PCR was the only assay with which illegitimate transcription in blood or sputum samples from healthy donors or patients with unrelated diseases did not interfere. However, it reproducibly detected up to 10 small-cell lung cancer cells diluted in either 10 ml blood or 5 ml sputum samples. Single blood and sputum samples were collected directly before diagnostic bronchoscopy from 175 patients suspected to have lung cancer. Twenty-six of these had small-cell lung cancer (SCLC). Thereof, 13 patients (50%) tested positive in the blood sample and 5 of 23 patients (22%) tested positive in the sputum sample. Moreover, among 92 patients with non-small-cell lung cancer (NSCLC) 25 patients (27%) had disseminated cancer cells in peripheral blood. Amplification of preproGRP transcripts from clinical samples is a sensitive and specific assay to detect disseminated or exfoliated lung cancer cells either in peripheral blood or sputum samples.

PMID: 11279599 [PubMed]

☐ **3:** Int J Cancer. 1995 Dec 11;63(6):810-4. Related Articles, Links

High frequency of K-ras mutations in normal appearing lung tissues and sputum of patients with lung cancer.

Yakubovskaya MS, Spiegelman V, Luo FC, Malaev S, Salnev A, Zborovskaya I, Gasparyan A, Polotsky B, Machaladze Z, Trachtenberg AC, et al.

Institute of Carcinogenesis, Russian Academy of Medical Sciences, Moscow, Russian Federation.

To evaluate the possible use of mutant ras as a biomarker for lung cancer, we have analyzed "normal appearing" lung tissue, lung tumor, lung metastases and sputum samples from patients with non-small cell lung cancer (NSCLC). As a control, we used lung tissue and sputum samples from patients without oncological diseases or lung disorders. Our analyses were performed with the aid of enriched PCR (EPCR), a method which enables detection of ras mutation even if present at low incidence. EPCR identified K-ras codon 12 mutations in 10% of lung tissues obtained from patients with no lung diseases, whereas the same mutation was detected in 60% of samples of normal appearing lung tissues obtained from patients with NSCLC, 62% of NSCLC tumors and 80% of metastases. Analysis of sputum samples of patients with NSCLC identified 47% to harbor mutant ras allele, whereas 12.5% of controls diagnosed with non-oncological lung diseases carried this mutation. Most of these mutations were detected with the aid of EPCR only, indicating that a minority of cells in a given sample harbor this mutation. The ability to detect K-ras codon 12 mutation in 60% of lung tissue samples and in 47% of sputum samples taken from patients with lung cancer (as compared with 10% and 12.5% of respective controls) points to the potential use of ras mutation as a biomarker for exposure and possible identification of patients who may be at higher risk of developing lung cancer.

PMID: 8847139 [PubMed]

Ai Zheng. 2002 May;21(5):533-5.

[Detection of telomerase hTERT gene expression of exfoliated cell in broncho-alveolar lavage fluid]

[Article in Chinese]

Ma G, Gao JS, Tong M, Xiong JG, He YS, Rong TH.

Cancer Hospital, Sun Yat-sen University, Guangzhou 510060, P. R. China.

BACKGROUND & OBJECTIVES: The patients with lung carcinoma usually have high telomerase activity, the pseudopositive result is happen frequently when the test is done with traditional method. Telomerase activity is more strongly correlated with hTERT mRNA. This study was designed to detect the expression of telomerase hTERT gene in broncho-alveolar lavage fluid of the patients with lung carcinomas by relative quantitative RT-PCR method, to afford a useful tool for early and differentiate diagnosis of lung cancer. METHOD: The exfoliated cells in broncho-alveolar lavage fluid from 20 human suspicion lung carcinomas and 10 health persons were analyzed for hTERT

expression by relative quantitative RT-PCR method, in the same time a part of exfoliated cells were detected with pathologic method. After operation, pathologic detection was performed for all samples. RESULTS: Of 20 human suspicion lung carcinomas and, 19 patients were lung cancer, 1 patient was inflammatory pseudotumor in pathological diagnosis after operation. The relative expression of telomerase hTERT gene in 16 cases of 19 patients with lung carcinoma were detected with the relative quantitative RT-PCR, the positive rate is 84.2% (16/19), the expressions of telomerase hTERT gene were at various quantities, average levels is 0.42. The positive rate in exfoliated cells detected with pathologic method were 57.9% (11/19). There was a statistical difference between them($P < 0.01$). Ten normal persons were hTERT gene expression negative. CONCLUSIONS: Relative quantitative RT-PCR were more sensitive than cell morphologic method in detecting telomerase hTERT gene expression in broncho-alveolar lavage fluid of patients with lung carcinomas. Detecting quantitatively the levels of telomerase hTERT gene expression in broncho-alveolar lavage fluid will may be useful for early and differentiate diagnosis of lung carcinomas, especially for peripheral lung carcinomas.

PMID: 12452048 [PubMed]

Gastric cancer by (RT-PCR) assay was used to detect carcinoembryonic antigen (CEA) mRNA in abdominal lavage fluid.

J Exp Clin Cancer Res. 2002 Dec;21(4):547-53. Related Articles, Links

Carcinoembryonic antigen mRNA in abdominal cavity as a useful predictor of peritoneal recurrence of gastric cancer with serosal exposure.

Fujii S, Kitayama J, Kaisaki S, Sasaki S, Seto Y, Tominaga O, Tsuno N, Umetani N, Yokota H, Kitamura K, Tsuruo T, Nagawa H.

Dept. of Surgical Oncology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

Peritoneal dissemination is the most frequent type of recurrence in patients with gastric cancer with serosal exposure, irrespective of whether they have undergone curative gastrectomy. The purpose of this study was to establish a method to detect micrometastatic cells in the abdominal cavity and predict peritoneal recurrence in patients with such gastric carcinomas. A total of 86 patients with gastric carcinoma, undergoing gastrectomy, were examined. Reverse transcriptase-polymerase chain reaction (RT-PCR) assay was used to detect carcinoembryonic antigen (CEA) mRNA in abdominal lavage fluid. Twenty-four cases without serosal exposure were negative, while all 13 cases with macroscopic peritoneal dissemination were positive for CEA mRNA. Among the 49 cases with macroscopic serosal invasion and without peritoneal metastasis, cancer cells were detected in 27 cases with RT-PCR while in only 6 cases with conventional

cytology. All cytologically-positive cases were also positive for CEA mRNA. Among the 27 CEA-positive cases, 15 patients (56%) relapsed with peritoneal metastasis within 12 months after gastrectomy. In contrast, none of the 22 CEA-negative cases had peritoneal recurrence within 16-60 months of observation, whereas in 43 cytologically-negative cases, 10 patients relapsed with peritoneal recurrence. As compared with conventional cytological examination, this method would be clinically more beneficial for detecting free cancer cells in the peritoneal cavity and for predicting peritoneal recurrence in gastric carcinoma with serosal invasion.

PMID: 12636101 [PubMed]

Prostate

Cancer Res. 2002 May 1;62(9):2695-8. Related Articles, Links

Comment in:

- Cancer Res. 2003 Aug 1;63(15):4747; author reply 4748-9.

DD3(PCA3), a very sensitive and specific marker to detect prostate tumors.

de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, Swinkels DW, Schalken JA.

Department of Clinical Chemistry, University Medical Centre Nijmegen, 6500 HB Nijmegen, The Netherlands.

We identified DD3(PCA3) as one of the most prostate cancer-specific genes at present (M. J. Bussemakers et al. Cancer Res., 59: 5975-5979, 1999). Consequently, DD3(PCA3) is an interesting candidate for use as a diagnostic and/or prognostic marker. In this study we developed a method for the accurate quantification of DD3(PCA3) mRNA, using real-time quantitative reverse transcription-PCR. DD3(PCA3) was expressed at low levels in normal prostate but not in 21 selected other normal tissues, blood, or 39 tumor samples other than prostate. The diagnostic and prognostic value of DD3(PCA3) in normal, hyperplastic, and malignant prostate tissues was determined and compared with another promising tumor marker for prostate cancer, telomerase reverse transcriptase (hTERT gene), the expression of which is related to telomerase activity. Sensitivity and specificity estimates for both genes were calculated as the area under the receiver-operating characteristics curve (AUC-ROC). DD3(PCA3) (AUC-ROC, 0.98) demonstrated better diagnostic efficacy than hTERT (AUC-ROC, 0.88). Moreover, the median increase in mRNA expression in tumor tissues compared with nonmalignant prostate tissues was much higher for DD3(PCA3) (34-fold) than for hTERT (6-fold). In

tumor tissues, the median expression of DD3(PCA3) was much higher than hTERT (5849 versus 10 normalized mRNA copies). A significant relationship was observed only between tumor stage and hTERT gene expression. We conclude that expression of the DD3(PCA3) gene is a very sensitive and specific marker for the detection of prostate tumor cells in a high background of normal (prostate) cells. Consequently, DD3 measurements may be used for clinical application in prostate needle biopsies or bodily fluids such as blood, ejaculate, urine, or prostate massage fluid.

PMID: 11980670 [PubMed]

Gan To Kagaku Ryoho. 2001 Jun;28(6):784-8. Related Articles, Links

[Rapid quantitative detection of free cancer cells in the peritoneal cavity of gastric cancer patients with real-time RT-PCR, and its prognostic significance]

[Article in Japanese]

Nakanishi H, Kodera Y, Yamamura Y, Tatematsu M.

Division of Oncological Pathology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan.

We have established a rapid and quantitative detection method using real-time RT-PCR on the LightCycler instrument. This method can reproducibly quantitate 10-10(5) CEA expressing colon carcinoma cells per 10(7) peripheral blood leukocytes, a sensitivity comparable with conventional RT-PCR with a wide linear measuring range. Analysis of peritoneal washes from 241 gastric cancer patients with this assay revealed relative values of CEA transcripts that correlate well with the depth of tumor invasion. The sensitivity and specificity of real-time RT-PCR with a cut-off value of 0.25 were 88% and 81%, respectively. At this cut-off value, patients in the real-time RT-PCR (+) group were found to suffer from peritoneal metastasis with a high frequency, while peritoneal recurrence was very rare among patients with real-time RT-PCR (-) results. These results indicate a positive correlation between CEA mRNA levels in peritoneal washes and patient prognosis. We conclude that real-time RT-PCR with hybridization probes is a sensitive, quantitative, specific and rapid method to detect free cancer cells in peritoneal washes.

Publication Types:

- Review
- Review Literature

PMID: 11432345 [PubMed]

Clin Cancer Res. 2001 Jun;7(6):1647-53.

Prediction of peritoneal micrometastasis by peritoneal lavaged cytology and reverse transcriptase-polymerase chain reaction for matrix metalloproteinase-7 mRNA.

Yonemura Y, Fujimura T, Ninomiya I, Kim BS, Bandou E, Sawa T, Kinoshita K, Endo Y, Sugiyama K, Sasaki T.

Second Department of Surgery, School of Medicine, Kanazawa University, Kanazawa 920-8640, Japan. yonemu@med.kanazawa-u.ac.jp

PURPOSE: Peritoneal dissemination is the most common cause of death associated with gastric cancer. In this study, we report the significance of molecular diagnosis of peritoneal dissemination by means of matrix metalloproteinase-7 (MMP-7) reverse transcriptase-PCR (RT-PCR) assay using preoperative peritoneal wash fluid. EXPERIMENTAL DESIGN: Preoperative peritoneal lavage by paracentesis was performed on 152 patients with gastric cancer. The peritoneal lavaged fluid was subjected to RT-PCR analysis with primers specific for MMP-7 and conventional cytological Papanicolaou examination. RESULTS: The MMP-7 RT-PCR assay was able to detect cancer cells at densities even lower than 10 cells/sample. There was no signal of MMP-7 mRNA from mesothelial cells, fibroblasts, peripheral blood, and lavaged fluid from patients with benign disease. Cytological examination and MMP-7 RT-PCR assay results were positive for 27 (18%) and 28 (18%) samples, respectively. The sensitivity for the prediction of peritoneal dissemination by cytology and MMP-7 RT-PCR assay were 46% and 33%, but the combination analysis using both parameters improved the sensitivity rate with 62%. Logistic regression analysis revealed that the cytological examination and MMP-7 RT-PCR assay are independent predictors of peritoneal dissemination. CONCLUSION: The combination of cytological examination and RT-PCR assay of preoperative peritoneal lavaged fluid is a highly efficient and reliable method for the selection of patients for adjuvant i.p. chemotherapy.

PMID: 11410502 [PubMed]

J Gastrointest Surg. 2001 Jan-Feb;5(1):66-73.

Laparoscopic-assisted vs. open colectomy for colorectal cancer: influence on neoplastic cell mobilization.

Bessa X, Castells A, Lacy AM, Elizalde JI, Delgado S, Boix L, Pinol V, Pellise M, Garcia-Valdecasas JC, Pique JM.

Department of Gastroenterology, Institut de Malalties Digestives, Hospital Clinic, Institut d'Investigacious Biomediques August Pi y Sunyer, University of Barcelona, Villaroel

170, 08036 Barcelona, Catalonia, Spain.

Laparoscopic surgery for treatment of colorectal cancer has been suggested to enhance tumor dissemination. Recently, molecular techniques have been developed to detect micrometastatic disease in patients with solid tumors, with a higher accuracy than cytologic or immunohistochemical approaches. This study was undertaken to investigate the potential harmful effects of laparoscopic-assisted colectomy on neoplastic cell mobilization in patients with resectable colorectal cancer. Fifty patients with nonmetastatic colorectal cancer were randomly assigned to laparoscopic-assisted (LAC, n = 26) or open (OC, n = 24) colectomy. Peripheral venous blood samples were obtained preoperatively, immediately after tumor removal, and 24 hours later. In 10 patients from each treatment group, portal blood and peritoneal fluid samples were also obtained before and after resection. Neoplastic cells were detected by means of reverse transcriptase-polymerase chain reaction targeted to carcinoembryonic antigen (CEA) transcription. CEA mRNA was detected in peripheral venous blood samples from 35 of 50 colorectal cancer patients preoperatively. Among those 15 baseline-negative patients, four experienced conversion 24 hours after tumor resection (2 [33%] of 6 in the LAC group vs. 2 [22%] of 9 in the OC group; NS). At that time point, clearance of CEA mRNA expression was observed in 14 of the 35 baseline-positive patients (9 [45%] of 20 in the LAC group vs. 5 [33%] of 15 in the OC group; NS). In addition, only one patient in the LAC group with baseline-negative CEA mRNA expression experienced portal blood conversion after tumor removal, although his peripheral blood level remained negative. Finally, baseline peritoneal fluid CEA mRNA expression was never detected, but one patient in each group became positive postoperatively. These results confirm that preoperative and perioperative mobilization of neoplastic cells is a frequent occurrence in patients with colorectal cancer, but the surgical approach (LAC vs. OC) does not seem to be a determining factor.

Publication Types:

- Clinical Trial
- Randomized Controlled Trial

PMID: 11309650 [PubMed]

Lung Cancer. 1998 Nov;22(2):153-6. Related Articles, Links

A case of leptomeningeal metastasis from lung adenocarcinoma diagnosed by reverse transcriptase-polymerase chain reaction for carcinoembryonic antigen.

Fujita J, Ueda Y, Bandoh S, Namihira H, Ishii T, Takahara J.

First Department of Internal Medicine, Kagawa Medical University, Japan.

A case of leptomeningeal metastasis from lung adenocarcinoma is reported. In this case, we evaluated the feasibility of reverse transcriptased polymerase chain reaction (RT-PCR) methods to detect cancer cells in cerebrospinal fluids (CSF). Messenger RNA of carcinoembryonic antigen (CEA) was clearly demonstrated in CSF by reverse RT-PCR methods. An immunohistochemical study also demonstrated that tumor cells were stained positive with anti-CEA antibody. This case suggests that RT-PCR for CEA was a sensitive and useful method to diagnose leptomeningeal metastasis from lung adenocarcinoma.

Publication Types:

- Case Reports

PMID: 10022223 [PubMed]

Anticancer Res. 2003 Mar-Apr;23(2B):1271-6. Related Articles, Links

Real-time PCR (TaqMan PCR) quantification of carcinoembryonic antigen (CEA) mRNA in the peripheral blood of colorectal cancer patients.

Miura M, Ichikawa Y, Tanaka K, Kamiyama M, Hamaguchi Y, Ishikawa T, Yamaguchi S, Togo S, Ike H, Ooki S, Shimada H.

Department of Surgery-II, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Japan, 236-0004. yasu0514@med.yokohama-cu.ac.jp

Reverse transcriptase-polymerase chain reaction (RT-PCR) has been utilized to detect living micrometastases of cancer cells in the lymph node, ascites or circulation system. However, the method was so sensitive that false-positives happened frequently. Therefore we have developed a quantification of CEA mRNA using real-time PCR to detect living cancer cells in the circulating blood and examined its usefulness as a predictive marker for liver metastases of colon cancer. In cell spiking experiments, it was possible to detect CEA mRNA in 10(1) cancer cells diluted in 10(7) normal lymphocytes. In the blood samples of cancer patients, the CEA mRNA level was significantly higher in Dukes' D patients than in the other clinical stages of colorectal cancer. This indicates that quantification of CEA mRNA is useful for the evaluation of colorectal cancer progress and that the post-operative increase of CEA mRNA can be a predictive marker for micrometastasis.

Publication Types:

- Evaluation Studies

PMID: 12820382 [PubMed]

Eur Urol. 2002 Jun;41(6):677-85. Related Articles, Links

Chorionic gonadotropin beta-subunit and core fragment in bladder cancer: mRNA and protein expression in urine, serum and tissue.

Hotakainen K, Haglund C, Paju A, Nordling S, Alfthan H, Rintala E, Stenman UH.

Biomedicum Helsinki, Room A418a, Helsinki University Central Hospital Research Laboratory, PB 700, 00029, HUCH, Finland. kristina.hotakainen@hus.fi

OBJECTIVES: Many transitional cell carcinomas (TCC) of the bladder express the beta-subunit (CGbeta) of chorionic gonadotropin (CG), and elevated serum levels occur especially in advanced disease. We have compared the diagnostic utility of various methods for detecting CG and CGbeta expression at the protein and mRNA level. METHODS: We used RT-PCR to detect CGbeta mRNA in urinary cells and highly sensitive immunoassays to determine CG and CGbeta in serum and the core fragment of CGbeta (CGbetacf) in urine from patients under follow-up for bladder cancer. Tissue expression was studied by immunohistochemistry. RESULTS: CGbeta mRNA was detected in urinary cells in 50% (n=84) of the cancer cases and in none of the healthy controls (n=15). Positive staining for CGbeta in tissue samples was observed not only in 30% (n=96) of the TCC cases, but also in 5 of 20 histologically benign samples from TCC patients, and in 10 of 21 samples from benign bladder diseases. Serum and urinary concentrations of CGbeta were elevated in 29% (n=66) and 8% (n=72), respectively, while serum CG was elevated in 18% of the TCC patients. Urinary CGbetacf concentrations were higher in invasive (T1-T4) than superficial (T in situ and Ta) tumors ($p=0.037$), in cases positive for CGbeta mRNA ($p=0.03$) and cases with suspicious or malignant urinary cytology ($p=0.002$). The ratio of urinary to serum concentration of CGbeta showed the strongest correlation with tumor stage ($p<0.00001$), grade ($p<0.00001$), and staining for CGbeta ($p=0.019$). CONCLUSIONS: Although CGbeta expression may occur in benign bladder epithelium, CGbeta mRNA in urinary cells is a potential marker of bladder cancer. Urinary and serum CGbeta have low sensitivity in early disease, but the urine/serum ratio appears to indicate local release of CGbeta into urine. Further studies are needed to evaluate the clinical usefulness of different forms of CGbeta expression.

PMID: 12074787 [PubMed]

J Urol. 2001 Dec;166(6):2134-41. Related Articles, Links

Real time reverse transcriptase polymerase chain reaction of urinary cytokeratin 20 detects transitional cell carcinoma cells.

Inoue T, Nakanishi H, Inada K, Hioki T, Tatematsu M, Sugimura Y.

Department of Urology, Aichi Cancer Center Hospital and Laboratory of Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan.

PURPOSE: We evaluate the diagnostic use of cytokeratin 20 messenger (m) RNA quantitation in urine as a marker of urothelial transitional cell carcinoma using the real time reverse transcriptase polymerase chain reaction (RT-PCR). MATERIALS AND METHODS: Spontaneously voided urine was obtained from 47 patients with urothelial transitional cell carcinoma (carcinoma group), 19 other urological diseases (noncarcinoma group) and 27 healthy volunteers (control group). Quantification of cytokeratin 20 was performed with mRNA extracted from urine samples with primers and hybridization probes specific for cytokeratin 20 on a LightCycler instrument (Roche Diagnostics Corp., Indianapolis, Indiana). RESULTS: This method allowed reproducible quantitation of 10 to 106 cytokeratin 20 expressing colon carcinoma cells per 107 peripheral blood leukocytes, comparable to the sensitivity of conventional RT-PCR with a wide linear measuring range. Cytokeratin 20 mRNA values in the carcinoma group (mean 35,850) were significantly higher than noncarcinoma (171) and control groups (4.55, $p < 0.0001$ and < 0.0001, respectively). Urinary cytokeratin 20 mRNA values significantly correlated with tumor grade, urinary cytological class, immunostaining pattern and depth of tumor invasion. Sensitivity and specificity of real time RT-PCR with a cutoff value of 15 were 81% and 83%, whereas those of conventional cytology were 28% and 100%, respectively. CONCLUSIONS: These results indicate that real time cytokeratin 20 RT-PCR is a sensitive, quantitative, rapid and specific method to detect free cancer cells in the urine, with good potential for monitoring recurrence of urothelial transitional cell carcinoma.

PMID: 11696722 [PubMed]

Int J Cancer. 1999 Jun 21;84(3):304-8. Related Articles, Links

Detection of messenger RNA for the beta-subunit of chorionic gonadotropin in urinary cells from patients with transitional cell carcinoma of the bladder by reverse transcription-polymerase chain reaction.

Hotakainen K, Lintula S, Stenman J, Rintala E, Lindell O, Stenman UH.

Department of Clinical Chemistry, Helsinki University Central Hospital, Finland. kristina.hotakainen@helsinki.fi

We studied whether detection of messenger-RNA (mRNA) for the beta-subunit of chorionic gonadotropin (CGbeta) in urinary cells from bladder cancer patients could be used as a marker of disease activity. Sixty-eight urine samples from patients under

follow-up for bladder cancer and 23 samples from patients with other malignancies and non-malignant surgical conditions, as well as 14 samples from healthy controls were analyzed. RNA was isolated from urinary cells collected by centrifugation. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect CGbeta mRNA. The results were compared to those obtained by cystoscopy and urinary cytology. For comparison, we determined CG and CGbeta in serum and urine and the core fragment of CGbeta (CGbeta cf) in urine by immunofluorometric assays. CGbeta mRNA was detected in 29 of 68 urine samples from patients with a history of bladder cancer, whereas all 14 samples from healthy controls tested negative. Elevated levels of CGbeta were observed in serum in 18 of 45 bladder cancer patients, but the association with CGbeta mRNA was weak. However, CGbeta mRNA expression in the absence of detectable cancer also occurred in some conditions associated with cellular atypia such as urinary tract infection, instrumentation and certain therapies. There was a highly significant association between histologically verified transitional cell carcinoma of the bladder and CGbeta mRNA in urine ($p = 0.0014$), implying CGbeta mRNA expression in tumor tissue. We conclude that CGbeta mRNA is a potential new marker for monitoring of bladder cancer. Further studies are needed to evaluate whether it provides independent clinical information.

PMID: 10371351 [PubMed]

Int J Cancer. 1994 Jun 1;57(5):671-5. Related Articles, Links

Neuroblastoma cell detection by reverse transcriptase-polymerase chain reaction (RT-PCR) for tyrosine hydroxylase mRNA.

Burchill SA, Bradbury FM, Smith B, Lewis IJ, Selby P.

Candlelighters Children's Research Laboratory, St. James' University Hospital, Leeds, UK.

The presence of tumour cells in peripheral blood of neuroblastoma patients is of considerable clinical importance. Nucleic acid amplification offers an opportunity to detect very small numbers of such cells, but in neuroblastoma a frequent specific abnormality in the tumour DNA suitable for this purpose has yet to be identified. To facilitate the detection of such cells we have developed RT-PCR using tyrosine hydroxylase (TH) as a tissue-specific target gene. TH mRNA was detected in 3 neuroblastoma cell lines and in all neuroblastoma tumours examined, but was undetectable in peripheral blood from children without neuroblastoma. The method was highly sensitive, detecting 1-10 neuroblastoma cells per 10(7) blood cells. Thirty blood samples from 24 patients were analysed and results were compared with known disease status. At diagnosis 4/7 patient blood specimens were positive; the four positive samples were from stage-4 patients. In blood samples from these patients 6-8 weeks after the initiation of treatment, TH mRNA was undetectable. Of 7 samples taken at the time of clinical relapse, 5 were positive; 4 of these were from patients with evidence of disseminating disease. Of 16 blood samples from disease-free patients, 14 were negative

and 2 were positive. One positive patient in this group subsequently had a clinical relapse. These results show that this technique is of value for detecting neuroblastoma cells in peripheral blood. The significance of these cells at diagnosis, during treatment or on follow-up requires further evaluation.

PMID: 7910809 [PubMed]

Cancer Epidemiol Biomarkers Prev. 1995 Sep;4(6):643-7. Related Articles, Links

Noninvasive detection of putative biomarkers for colon cancer using fecal messenger RNA.

Davidson LA, Jiang YH, Lupton JR, Chapkin RS.

Faculty of Nutrition, Texas A&M University, College Station 77843-2471, USA.

Deaths from colon cancer number over 60,000 each year in the United States. Because early detection results in a high cure rate, development of noninvasive techniques for detection of colon cancer has received much interest. The ability to detect early changes in colonocyte genes and gene expression would provide valuable information. We have shown previously that alterations in protein kinase C (PKC) isoform expression are associated with changes in colonic cell proliferation, a key intermediate marker for the prediction of tumorigenesis. Here, we describe a method for the quantitative detection of mRNAs for select PKC isoforms isolated from rat feces containing exfoliated colonocytes. After total RNA extraction from fresh fecal material, polyadenylated RNA was selectively purified and quantitated with slot blotting and hybridization to oligodeoxythymidylic acid. Fecal polyadenylated RNA was used for semiquantitative (mimic) RT-PCR to quantitate PKC isoform mRNA expression. We detected mRNA for PKC-alpha, PKC-delta, PKC-epsilon, and PKC-sigma, but not for PKC-beta or PKC-gamma, which is consistent with the profile of isoforms detected previously in scraped colonic mucosa using immunoblot analysis. This noninvasive method, utilizing feces containing exfoliated colonocytes, is a sensitive noninvasive technique for quantitating luminal mRNAs. This provides a means to monitor gene expression of colonic epithelial cells, which may have predictive value in monitoring the neoplastic process.

PMID: 8547831 [PubMed]

Oral Oncol. 2000 May;36(3):272-6. Related Articles, Links

Mutation of p53 gene codon 63 in saliva as a molecular marker for oral squamous cell carcinomas.

Liao PH, Chang YC, Huang MF, Tai KW, Chou MY.

Department of Dentistry, Chung Shan Medical and Dental College Hospital, Taichung,

Taiwan.

The inactivation of tumor suppressor gene (TSG) is important during multistage carcinogenesis. The p53 TSG is frequently mutated in oral squamous cell carcinomas. These mutations can serve as very specific markers for the presence of tumor cells in a background of normal cells. In this study, 10 oral squamous cell carcinoma patients and 27 normal dental students were collected from Chung Shan Medical and Dental College Hospital, Taichung, Taiwan. Extractions of DNA from saliva were obtained. Exon 4 and intron 6 within the p53 gene were amplified with polymerase chain reactions (PCRs) followed by DNA sequence analysis. DNA sequence analysis of PCR products revealed that five of eight (62.5%) tumor saliva samples and five of 27 (18. 52%) healthy saliva samples contained p53 exon 4 codon 63 mutations. These results were significantly different by using Chi-square test ($P<0.05$). The majority of the base substitutions were C deletions. Probable hot spots for the mutation were identified at exon 4 codon 63, which has not been observed before in head and neck cancers. Our study indicated that mutation of p53 codon 63 in saliva might be a molecular marker for oral squamous cell carcinomas. In addition, the amount of DNA recovered from saliva in most cases is sufficiently large and its quality suitable to enable PCR amplification which could be used in the search for mutations. The protocol described is rapid, cheap, and easy to perform, and may be useful for epidemiological studies for oral carcinogenesis.

PMID: 10793330 [PubMed]